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sequence of a gene for Fanconi's anemia, thalassaemias, cystic fibrosis, sickle cell anemia, retinitis pigmentosa, xeroderma pigmentosum, ataxia telangiectasia, Bloom's syndrome, retinoblastoma, Duchenne's muscular dystrophy, or Tay-Sachs disease, with an exogenous replacement DNA fragment comprising 3' and 5' flanking intronic sequences homologous to 3' and 5' flanking sequences of the mutant DNA sequence, said intronic sequences adjacent to an exon carrying a silent mutation that gives rise to a novel restriction enzyme cleavage site not present in the exon carrying the targeted mutant DNA sequence, and the sequence to replace the targeted mutant DNA sequence, said method comprising steps:

(a) generating the exon containing targeted mutant DNA sequence to be replaced and 3' and 5' flanking intronic sequences adjacent to said exon;

(b) generating the exogenous replacement DNA fragment comprising homologous 3' and 5' flanking intronic sequences adjacent to the exon carrying a silent mutation that gives rise to a novel restriction enzyme cleavage site not present in the exon carrying the targeted mutant DNA sequence;

wherein a total size of the replacement DNA fragment is from 1 to 2000 bases;

wherein the replacement DNA fragment is a double stranded DNA generated of double stranded fragment by denaturation or by separation of a biotin labeled complementary strand from a strand

comprising the replacement fragment;

wherein the double stranded fragment is comprised of two complementary DNA strands each of which is able to replace one strand of the targeted genomic DNA;

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(c) transfecting or delivering the exogenous replacement DNA fragment of step (b) into the target cells containing the targeted mutant DNA sequence, wherein the transfection or the fragment delivery is accomplished by incubating the replacement DNA fragment of step (b) in a transfection cocktail, forming a transfection mix, introducing the incubated transfection mix to the cells of step (c) grown to 70-90% confluence, and incubating the transfection mix in the presence of the cells at about 32 to 40°C temperature;

(d) propagating the incubated cells in a fresh growth medium for about two days to about two weeks; and

(e) determining the extent of homologous replacement by PCR identification of cells within the total cell population which have replaced the endogenous targeted mutant DNA with the exogenous replacement DNA fragment at a target genomic locus.

9. (New) The method of claim 8 wherein said replacement DNA fragment sequence is identified by primers of about 25 bases that are outside of regions of homology defined by exogenous replacement DNA fragment, or primers that are allele-specific and differentiate between the endogenous targeted mutant sequence and the exogenous replacement DNA fragment, or by Southern hybridization with allele-

specific oligonucleotide probes that differentiate between the endogenous targeted mutant sequence and the exogenous replacement DNA fragment.

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10. (New) The method of Claim 9 wherein said replacement DNA fragment is generated by PCR amplification, oligonucleotide synthesis, plasmid cleavage with restriction endonuclease or by a combination of restriction enzyme cleavage of plasmid inserts and ligation of contiguous insert fragments.

11. (New) The method of claim 10 wherein said replacement DNA fragment is uncoated or coated with a recombinase or complexed with a protein, provided that when the DNA fragment is coated with recombinase is not recA.

12. (New) The method of claim 11 wherein the exogenous replacement DNA fragment of step (b) is delivered into the target cell by electroporation, microinjection, complexed onto a lipid layer, in a dendrimer or conjugated to polylysine.

13. (New) The method of claim 12 wherein the targeted mutant DNA sequence to be replaced is a DNA sequence present in the cystic fibrosis gene and wherein the primers are selected from the group consisting of primers CF1, CF1B, CF5, CF6, CF7B, CF8B, CF7C, CF8C, CF9, CF14, CF17 and CF22.

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14. (New) The method of claim 12 wherein the targeted mutant DNA sequence to be replaced is a DNA sequence present in the sickle cell anemia gene wherein said sequence is replaced with a replacement genomic DNA sequence encoding β -globin, said genomic DNA further containing non-coding sequences flanking the sequences to be replaced, and wherein the primers are selected from the group consisting of primers SC1(+), SC2(-), SC3(+), SC4(-), SC5(+), SC6(-), SC-BA(-) and SC-BS(-).

15. (New) The method of claim 12 wherein the targeted mutant DNA sequence to be replaced is a DNA sequence present in the gene causing thalassaemias, wherein said sequence is replaced with a replacement genomic DNA sequence in the thalassaemias causing genomic locus, said replacement DNA sequence further containing non-coding sequences flanking the replacement sequence.

16. (New) The method of claim 12 wherein the targeted mutant DNA sequence to be replaced is a DNA sequence present in a gene causing xeroderma pigmentosum, wherein said sequence is replaced with a replacement genomic DNA that contains non-coding sequences flanking the replacement sequence.

REMARKS

This Amendment and Response is being filed in response to the Examiner's Restriction Requirement dated July 21, 2000.